In accordance with this embodiment, the present method is used to deliver a DNA sequence or an RNA sequence, including recombinant genes, to tumor cells in vivo with (1) retroviral or viral vectors as vehicles, (2) DNA or RNA/liposome complexes as vehicles, (3) chemical formulations containing the DNA or RNA sequence and coupled to a carrier molecule which facilitates delivery of the sequence to the targeted cells, or (4) by utilizing cell-mediated gene transfer to deliver genes to specific sites in vivo, e.g., by relying upon the use of vascular smooth muscle cells or endothelia cells which have been transduced in vitro as a vehicle to deliver the recombinant gene into the site of the tumor.

In an aspect of this embodiment, the present invention relies on the immune system to provide protection against cancer and play an important role as an adjuvant treatment for a malignancy. Immunotherapy has shown promise as an adjuvant approach to the treatment of malignancies. Both cytolytic T cells and lymphokines can facilitate tumor cell destruction, and strategies to enhance tumor regression by administration of cytokines or tumor infiltrating lymphocytes have shown efficacy in animal models and human trials. For example, it is known that lymphokine activated killer cells (LAK) and tumor infiltrating lymphocytes (TIL) can lyse neoplastic cells and produce partial or complete tumor rejection. Expression of cytokine genes in malignant cells has also enhanced tumor regression.

The present invention provides a novel gene transfer approach against tumors by the introduction of recombinant genes directly into tumor cells _in vivo_, where, by contrast, traditional gene transfer techniques have focused on modification of tumor cells _in vitro_ followed by transfer of the modified cells. The prior art approaches are disadvantageous because they subject the cells to selection in different growth conditions from those which act _in vivo_, and because they also require that cell lines be established for each malignancy, thereby rendering adaptability to human disease considerably more difficult.

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Genes which may be used with this embodiment include genes containing a DNA sequence (or the corresponding RNA sequence may be used) encoding an intracellular, secreted, or cell surface molecule which is exogenous to the patient and which (1) is immunogenic to the patient, (2) induces rejection, regression, or both, of the tumor, or (3) is toxic to the cells of the tumor.

The vectors containing the DNA sequence (or the corresponding RNA sequence) which may be used in accordance with the invention may be an eukaryotic expression vector containing the DNA or the RNA sequence of interest. Techniques for obtaining expression of exogenous DNA or RNA sequences in a host are known. See, for example, _Korman et al_, _Proc. Nat. Acad. Sci. (USA)_ (1987) _84_:2150-2154, which is hereby incorporated by reference.

This vector, as noted above, may be administered to the patient in a retroviral or other viral vector (i.e., a viral vector) vehicle, a DNA or RNA/liposome complex, or by utilizing cell-mediated gene transfer. Further, the vector, when present in non-viral form, may be administered as a DNA or RNA sequence-containing chemical formulation coupled to a carrier molecule which facilitates delivery to the host cell. Such carrier molecule would include an antibody specific to the cells to which the vector is being delivered or a molecule capable of interacting with a receptor associated with the target cells.

Cell-mediated gene transfer may be used in accordance with the invention. In this mode, one relies upon the delivery of recombinant genes into living organisms by transfer of the genetic material into cells derived from the host and modification in cell culture, followed by the introduction of genetically altered cells into the host. An illustrative packaging cell line which may be used in accordance with this embodiment is described in Danos et al, Proc. Natl. Acad. Sci. (USA) (1988) 85:6460, which is hereby incorporated by reference.

The DNA or RNA sequence encoding the molecule used in accordance with the invention may be administered to the patient, which may be human or a non-human animal, either locally or systemically. The systemic administration is preferably carried out using the non-viral DNA or RNA chemical

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formulation coupled to a carrier molecule which facilitates delivery to the host cells. Any of the administrations may be performed by IV or IM injection or subcutaneous injection using any known means, or by the use of the catheter in accordance with the present invention.

The retroviral vector vehicles used in accordance with the present invention comprise a viral particle derived from a naturally-occurring retrovirus which has been genetically altered to render it replication defective and to express a recombinant gene of interest in accordance with the invention. Once the virus delivers its genetic material to a cell, it does not generate additional infectious virus but does introduce exogenous recombinant genes to the cell.

In other viral vectors, the virus particle used is derived from other naturally-occurring viruses which have been genetically altered to render them replication defective and to express recombinant genes. Such viral vectors may be derived from adenovirus, papillomavirus, herpesvirus, parvovirus, etc.

The sequences of the present invention may also be administered as DNA or RNA/liposome complex. Such complexes comprise a mixture of fat particles, lipids, which bind to genetic material, DNA or RNA, providing a hydrophobic coat, allowing genetic material to be delivered into cells. This formulation provides a non-viral vector for gene transfer. Liposomes used in accordance with the invention may comprise

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DOPE (dioleyl phosphatidyl ethanol amine), CUDMEDA (N-(5-cholestrum-3-β-ol 3-urethanyl)-N′, N′-dimethlethylene diamine).

As noted above, other non-viral vectors may also be used in accordance with the present invention. These include chemical formulations of DNA or RNA coupled to a carrier molecule (e.g., an antibody or a receptor ligand) which facilitates delivery to host cells for the purpose of altering the biologic properties of the host cells. The term "chemical formulations" used herein refers to modifications of nucleic acids to allow coupling of the nucleic acid compounds to a protein or lipid, or derivative thereof, carrier molecule. Such carrier molecules include antibodies specific to the host cells or receptor ligands, i.e., molecules able to interact with receptors associated with the host cells.

The molecules which may be used in accordance with this invention, include the following: (1) genes encoding immune stimulants, such as Class I histocompatibility genes, Class II histocompatibility genes, bacterial genes, including mycobacterial (PPD) genes and genes encoding heat shock proteins, viral glycoproteins encoding genes, including vesicular stomatitis virus G protein, influenza hemagglutinin, and herpes virus glycoprotein β, minor histocompatibility antigens, foreign proteins, such as lysozyme or bovine serum albumin, and oncogenes, including EIA, P53 (mutants) and tax; (2) immune and growth stimulants/inhibitors, including

inducers of differentiation, such as stimulants, including interleukin-2 (IL-2) IL-4, 3, 6 or 8, inhibitors/inducers of differentiation, such as TNF-α or β, TGF-β (1, 2 or 3), IL-1, soluble growth factor receptors (PDGF, FGF receptors), recombinant antibodies to growth factors or receptors, analogs of growth factors (PDGF, FGF), interferons (α, β or γ) and adhesion molecules; or (3) toxins or negative selectable markers, including thymidine kinase, diphtheria toxin, pertussis toxin or drug-sensitive proteins.

The DNA/RNA sequence is preferably obtained from a source of the same species as the patient, but this is not absolutely required, and the present invention provides for the use of DNA sequences obtained from a source of a species different from the patient in accordance with this embodiment. A preferred embodiment of the present invention, genes encoding immune stimulants and toxins or negative selectable markers, corresponding to (1) and (3) above, are preferably selected from a species different than the species to which the patient belongs. For immune and growth stimulants/inhibitors, corresponding to (2) above, in accordance with another preferred embodiment of the invention, one preferably employs a gene obtained from a species which is the same as the species of the patient.--

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IN THE CLAIMS